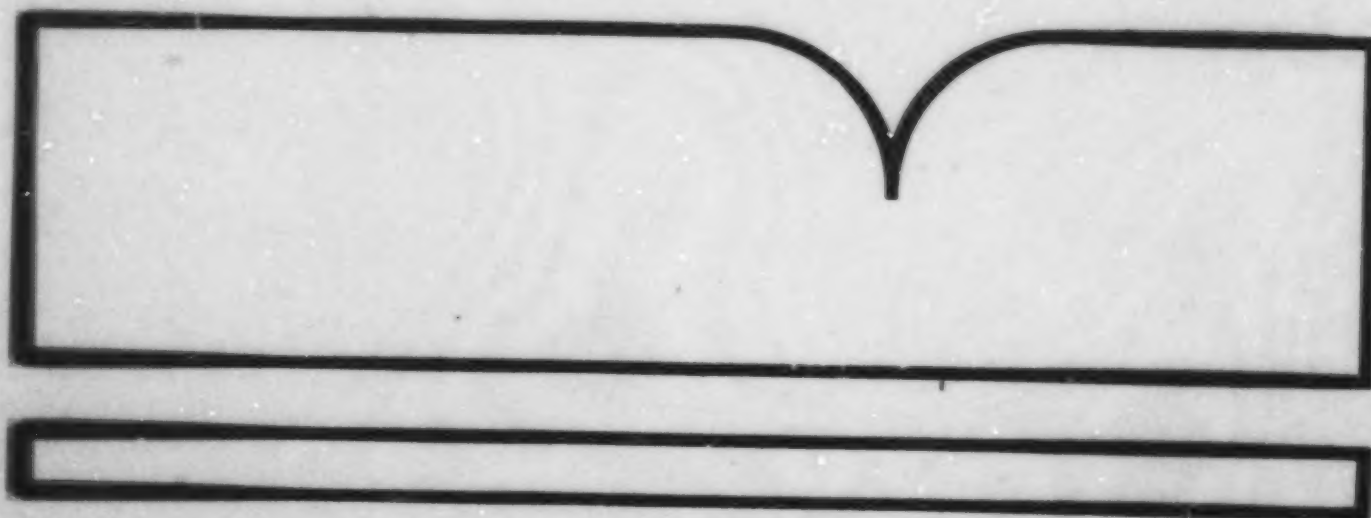


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Ventilatory Frequency of Mouse and
Hamster during Microwave-Induced Heat Exposure

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VENTILATORY FREQUENCY OF MOUSE AND HAMSTER DURING MICROWAVE-INDUCED HEAT EXPOSURE

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Abstract. Ventilatory frequency was recorded in unrestrained mice and hamsters using microwave radiation. The microwave exposure system was also used to impart varying heat loads to the rodents at ambient temperatures of 10, 20 and 30 °C. The absorbed heat load accrued from microwave exposure was measured as the time-averaged rate of energy absorption per unit body weight or specific absorption rate (SAR, W/kg). In both species there was an inverse relationship between ambient temperature (T_a) and threshold SAR to invoke an increase in ventilatory frequency. However, the threshold SAR's for mice were 270 to 450% higher than for hamsters depending on T_a . Above threshold SAR the increase in ventilatory frequency per unit elevation in SAR increased directly with T_a in mice but not with hamsters. On the basis of rate of absorbed energy normalized to body weight (*i.e.*, W/kg), mice can tolerate much more heat from microwave exposure than hamsters. The differences in sensitivity to microwave exposure in the mouse and hamster are probably attributable to species-specific characteristics, and especially differences in body mass and, consequently, passive heat loss.

Breathing frequency	Mouse
Hamster	Temperature
Microwave technique	

Rodents are generally considered to have a poor ability to dissipate heat through respiratory water loss. However, when the magnitude of heat stress is sufficient, rodents will increase their ventilatory frequency and, consequently, pulmocutaneous evaporative heat loss (Hart, 1971).

Recently our laboratory has developed a method for monitoring the ventilatory frequency of unrestrained rodents using microwave irradiation (Gordon, 1983b; Gordon and Ali, 1984). This tool provides an excellent means to study the effects of heat stress on breathing activity of rodents. The intensity of microwave radiation used to monitor ventilatory frequency can be controlled which permits exposing the animal to varying heat loads. Further, with this microwave exposure system the quantity and rate of heat absorption can be measured accurately (Gordon,

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1982). Most importantly, the study can be performed in unrestrained animals thereby eliminating stress factors that might affect the sensitivity of the thermoregulatory system (McEwen and Heath, 1973).

The sensitivity of thermoregulatory motor systems to ambient temperature has been reported for an array of species (Hammel, 1968; Cabanac, 1975). Heating of the preoptic/anterior hypothalamus (McEwen, 1973; McEwen and Heath, 1970; Inomoto *et al.*, 1983) and spinal cord (Guieu and Hardy, 1970) will evoke an increase in ventilatory frequency. Presumably, local warming of thermal sensitive sites in the central nervous system excites heat loss pathways resulting in an elevation in the rate of ventilation.

The microwave exposure system provides a means to assess the characteristics of the thermoregulatory control of ventilatory frequency. Since microwaves at a frequency of 2450 MHz are predominantly absorbed by the internal tissues of small rodents, the temperature-sensitive sites within and outside the central nervous system can be heated with microwaves while ambient temperature is maintained constant.

The purpose of this study is to record ventilatory frequency as a function of microwave thermal load in unrestrained mice and hamsters exposed to 2450 MHz microwaves at ambient temperatures of 10, 20 and 30 °C.

Materials and methods

Animals used in this study were six male BALB/C albino mice with a body weight of 24.3 ± 1.4 (SD) g and six male golden hamsters with a body weight of 106.0 ± 11.7 g. The animals were maintained at an ambient temperature (T_a) of 22 °C under a 12:12 L:D photoperiod, and given food and water *ad libitum*.

Breathing rate. The method of measuring ventilatory frequency using microwave radiation has been described in detail in the previous paper (Gordon and Ali, 1984) and will be explained briefly. As an animal inhales and exhales, its volume and, hence, efficiency to absorb microwaves changes concomitantly. In a waveguide exposure system used for the transmission of microwave energy, breathing activity by an animal causes small changes in unabsorbed microwave power passing through the waveguide. The DC analog output of a power meter monitoring the unabsorbed or transmitted power is differentially amplified and monitored on a chart recorder. Normally, if the animal is quiescent its breathing movements are readily detected by observing the rhythmic changes in transmitted power. However, any quick movement by the animal causes a change in transmitted power far greater than that of breathing resulting in temporary loss of the breathing record.

The dose of microwave energy, specific absorption rate (SAR), expressed in units of Joules absorbed per second per unit body weight (or W/kg), was measured as described previously (Gordon, 1982). SAR was recorded digitally every 2 min and then averaged over each 30 min exposure period.

Protocol. Mice and hamsters were singly exposed to 2450 MHz microwave radiation in a waveguide as described previously (Gordon, 1982). The body of the waveguide was immersed in a temperature-controlled water bath. Both mice and hamsters were exposed to microwaves at T_a values of 10, 20 and 30°C. Dry air was continually circulated through the waveguide.

After a 1 h accommodation period at a given T_a the animal was exposed to one incident power of microwave energy for 30 min. At the end of 30 min the incident power was increased to another level, generally by at least 50%, and exposure continued for another 30 min. Ventilatory frequency data were taken during the last 10 min of the first and second 30 min exposure periods. After microwave exposure the animal was allowed at least 48 h before being subjected to another experiment.

The intensity of microwave exposure was varied with species and T_a . The highest exposure intensities were made at the lowest T_a 's. For example, the maximum specific absorption rate (SAR) at a T_a of 10°C for mice reached approximately 140 W/kg whereas for hamsters at 10°C, maximum SAR was approximately 40 W/kg.

Baseline breathing, defined as ventilatory frequency of animals not affected by microwave heat stress, was determined by measuring ventilation rate during the first 60 sec of microwave exposure. During this period ventilatory frequency of the animals had not reacted to the thermal stress of microwave exposure.

Data analysis. The recorder tracings of breathing activity were analyzed by an impartial observer. Segments of data ranging from 10 to 30 sec were counted and averaged to calculate ventilatory frequency (breaths/min). This procedure was generally performed twice on each 10 min recording. A plot of ventilatory frequency vs SAR was developed for each species at a given T_a . Functional relationships between SAR and ventilatory frequency were expressed by linear regression. Correlation coefficients and 95% confidence intervals of the regression lines were computed assuming that data collected repeatedly from each animal were independent.

Results

Examples of breathing activity monitored via recording the differential of transmitted power with time in a hamster at a T_a of 20 and 30°C is shown in fig. 1. Note the relatively slow breathing pattern when exposed to 15.4 W/kg at 20°C compared to the more rapid breathing when exposed to 10.5 W/kg at 30°C.

Ventilatory frequency vs SAR at 10°C was highly variable for mice (fig. 2). In spite of the variability it is apparent that breathing rate underwent a sharp increase at an SAR $\approx$ 100 W/kg. Two deaths from microwave exposure, the only lethality in the study, occurred at 10°C during exposure to SAR's of 119 and 138 W/kg. The two animals survived the initial microwave exposure but were found dead in

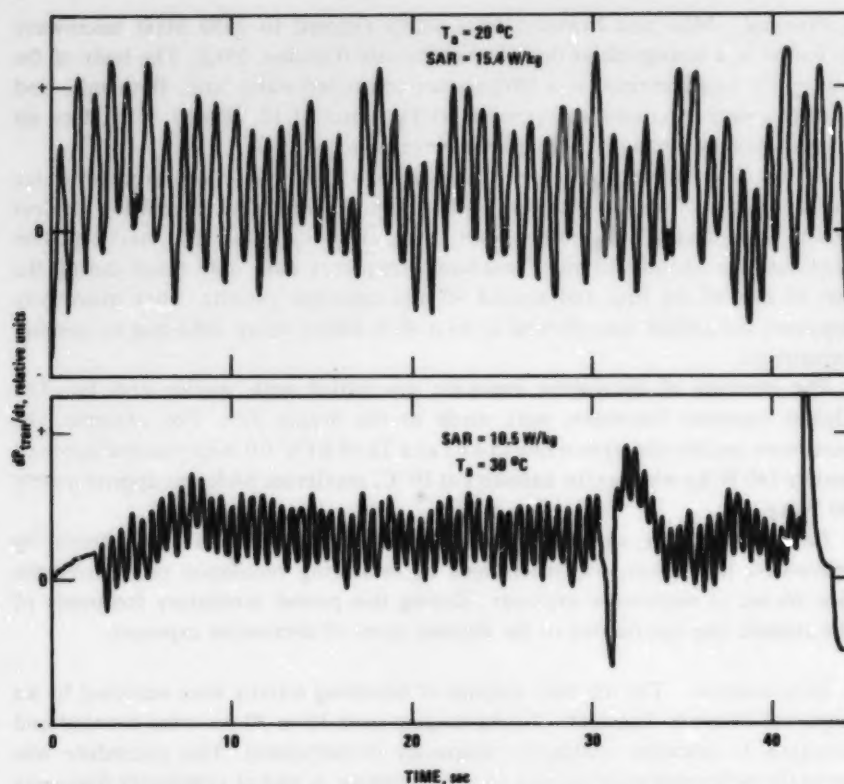


Fig. 1. Recording of the differential of transmitted power with time during exposure of a hamster at 2450 MHz microwaves in a waveguide maintained at an ambient temperature (T_a) of 20 and 30 °C. SAR is the specific absorption rate.

their cage the following day. The breathing rates of these animals were 185 and 150 breaths/min, respectively, which were not exceedingly high considering that the breathing rate of many of the mice exceeded 200 breaths/min.

Ventilatory frequency of mice at 20 °C also exhibited high variability, however, at this ambient temperature there was a clearer relationship between SAR and breathing activity. Ventilatory frequency increased when the SAR exceeded approximately 50 W/kg. At a T_a of 30 °C, ventilatory frequency underwent a linear increase at SAR's greater than approximately 10 W/kg. At T_a 's of 10 and 20 °C ventilatory frequency was elevated at exposure to low SAR's and then decreased slightly with increasing SAR up to a threshold, at which point ventilatory frequency increased.

The ventilatory response of the hamsters was less variable compared to the mice (fig. 3). At a T_a of 10 °C ventilatory frequency was relatively high at low SAR's,

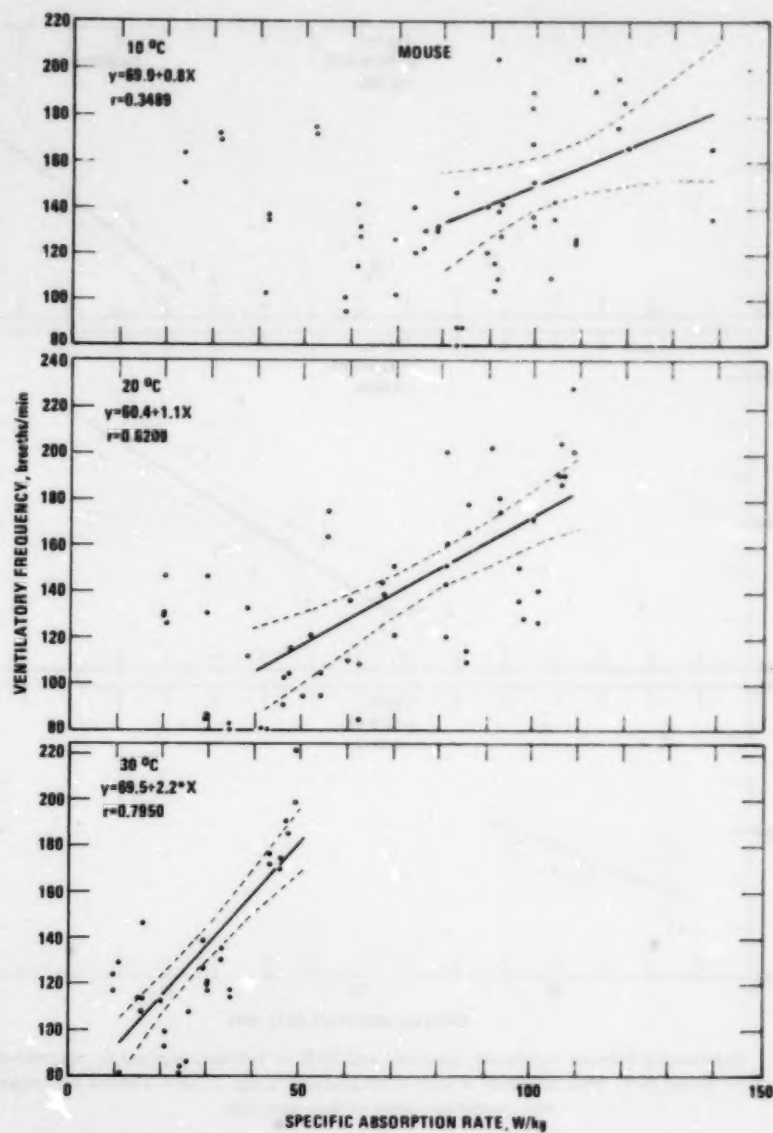


Fig. 2. Relationship between ventilatory frequency and SAR of mice exposed to microwaves at T_a 's of 10, 20 and 30 °C. Note reduction in threshold and increase in slope of regression line with increasing ambient temperature. Dashed lines represent 95% confidence limits of regression line.

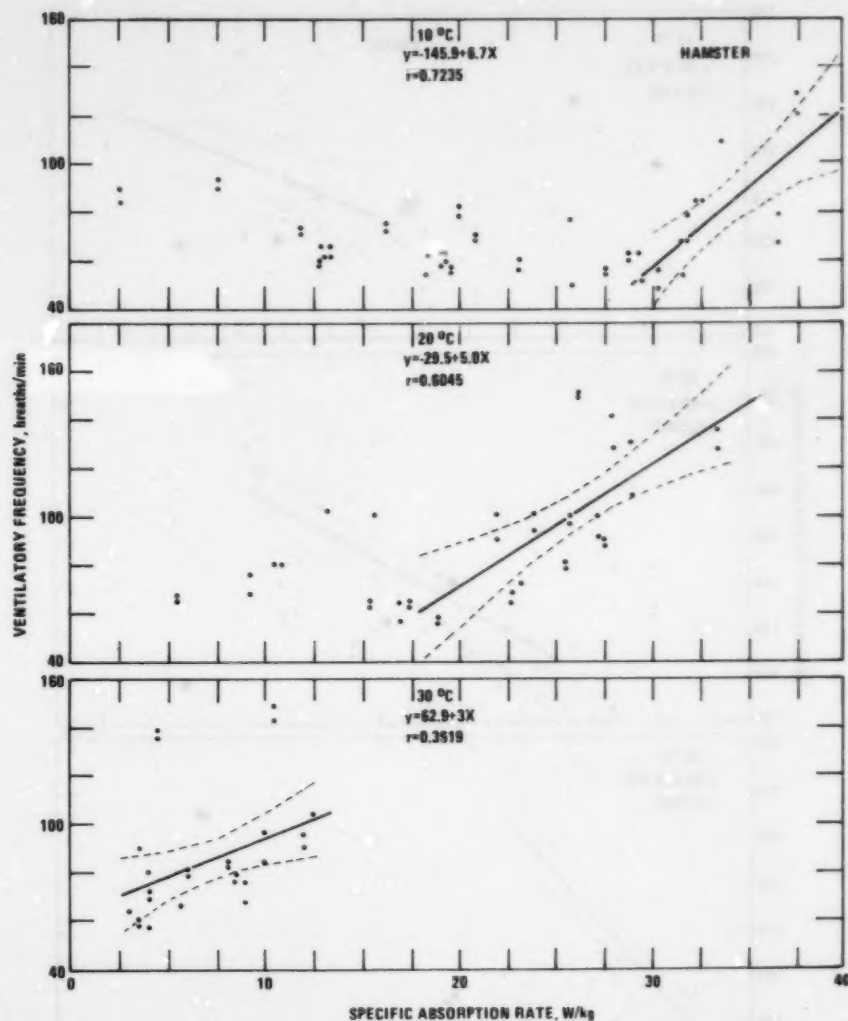


Fig. 3. Relationship between ventilatory frequency and SAR of hamsters exposed to microwaves at Ta's of 10, 20 and 30°C. Note difference in scale of the abscissas in figs. 2 and 3. Dashed lines represent 95% confidence limits of regression line.

gradually decreased with increasing SAR up to 30 W/kg and then increased abruptly above an SAR of 30 W/kg. At 20°C ventilatory frequency remained near baseline up to an SAR of 18 W/kg and then increased sharply with increasing SAR. At a Ta of 30°C ventilatory frequency increased at an SAR of approximately 2 W/kg.

Baseline ventilatory frequency, recorded during the first ≈ 60 sec of microwave exposure, was inversely dependent on T_a in mice but not hamsters (fig. 4). At a T_a of 30°C baseline ventilatory frequency of mice was 116 breaths/min and increased to 141 breaths/min at a T_a of 10°C . On the other hand, hamsters at 30°C had a baseline ventilatory frequency of 71 breaths/min which increased to only 76 breaths/min at a T_a of 10°C .

Discussion

Compared to other thermoregulatory effectors, ventilatory frequency has a low sensitivity to microwave radiation. For example, SAR's at 2450 MHz of ≈ 11 W/kg are sufficient to promote peripheral vasodilation in the tail of mice at a T_a of 20°C (Gordon, 1983a). At their normally preferred ambient temperature of 30 – 31°C , hamsters (Gordon and Long, 1984) and mice (Gordon, 1983b) will behaviorally select at cooler T_a when SAR exceeds 2.0 and 7.0 W/kg, respectively. Thus, the threshold SAR's to elevate ventilatory frequency in mice and hamsters are relatively high compared to other thermoregulatory motor outputs. It is likely that when ventilatory frequency does increase during microwave thermal stimulation the rodent can be considered excessively heat stressed and has activated all other thermoregulatory systems.

The present study has shown that the sensitivity (*i.e.* threshold) of the control of ventilation to a microwave-induced heat load is influenced by ambient temperature. In spite of the high variability occurring at some T_a 's, it is apparent that in both mouse and hamster the threshold SAR to induce an increase in ventilatory frequency decreased sharply with increasing T_a . Since the effectiveness of heat loss by convection, conduction, and radiation decreases as the gradient between skin and ambient temperature lessens, heat loss by respiratory evaporation plays a significantly greater thermoregulatory role at high ambient temperatures. The inverse relation between sensitivity of a heat dissipating thermoregulatory motor output (*i.e.*, ventilatory frequency) and ambient temperature is in line with past studies where effector activity was stimulated with thermodes stereotactically implanted in the preoptic area/anterior hypothalamus and other temperature sensitive sites in the central nervous system (Hammel, 1968; Cabanac, 1975). For example, respiratory heat loss in the dog increases abruptly at a threshold hypothalamic temperature which varies inversely with ambient temperature (Hellstrom and Hammel, 1967). The threshold hypothalamic temperature for increasing ventilatory frequency in the rabbit is nearly constant regardless of ambient temperature, however, the slope of the response (*i.e.*, breaths $\cdot (\text{min} \cdot ^\circ\text{C})^{-1}$) increases directly with ambient temperature (McEwen and Heath, 1975; McEwen, 1973).

In the present study ambient temperature and species influenced the threshold SAR and the slope of the response (*i.e.*, change in ventilatory frequency per unit increase in SAR above threshold). The threshold SAR for mice had a relatively sharp drop with increasing ambient temperature compared to the hamsters. The

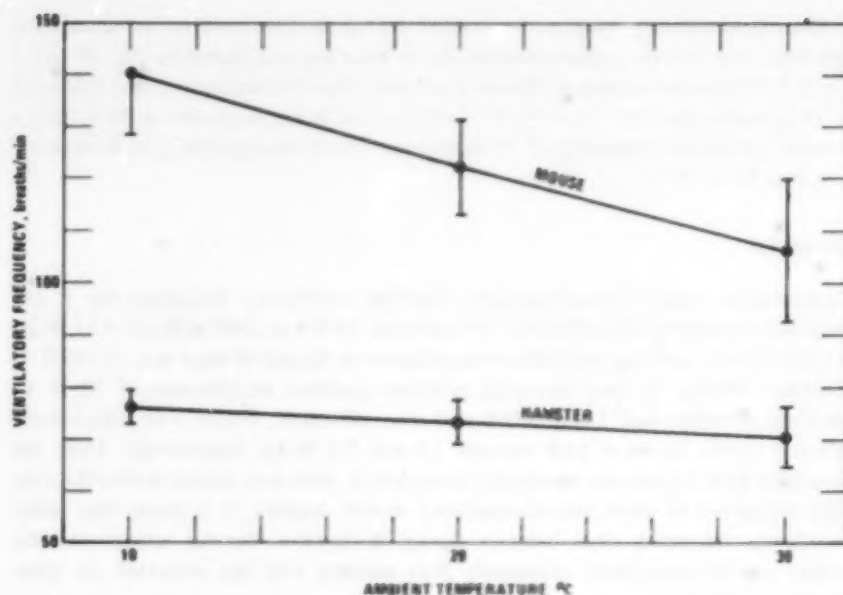


Fig. 4. Relationship between baseline ventilatory frequency (breathing recorded during first 60 sec of microwave exposure) and ambient temperature. Vertical bars represent 1 SEM.

slope increased gradually with ambient temperature in mice but decreased with increasing ambient temperature in hamsters. The effect of ambient temperature on the threshold and slope of a thermoregulatory effector as a function of hypothalamic temperature has been shown to be unique to a given species (Heath *et al.*, 1972).

Baseline ventilatory frequency was inversely dependent on T_a in mice but not hamsters (fig. 4). Assuming that ventilatory frequency is proportional to metabolic demand, then it appears that hamsters were less sensitive to reduced T_a compared with mice. This is unexpected since the hamster and mouse have similar lower critical ambient temperatures, 28 and 30 °C, respectively (Hart, 1971).

The mouse and hamster differed greatly in their overall sensitivity to a microwave exposure-induced increase in ventilatory frequency. For example, at a T_a of 20 °C an SAR of 50 W/kg was necessary to raise ventilatory frequency of mice, whereas hamsters at 20 °C could not possibly tolerate 50 W/kg. These differences, while perhaps attributable to unique characteristics of the species, are possibly a result of the large difference in body mass and surface area of the mouse and hamster. It is well known that, for most homeotherms, an increase in body mass of 1000 g is associated with a 10 fold drop in weight-specific thermal conductance (Aschoff, 1981). Based on an allometric scaling equation, the 106 g hamster has a predicted minimal thermal conductance of $0.5 \text{ W} \cdot (\text{kg} \cdot ^\circ\text{C})^{-1}$ while the 24 g mouse has a conductance of $1.0 \text{ W} \cdot (\text{kg} \cdot ^\circ\text{C})^{-1}$. (Aschoff, 1981).

A higher thermal conductance is indicative of a greater capacity to dissipate heat passively without using active physiological mechanisms. For example, Taylor (1977) calculated the maximum amount of non-evaporative heat loss relative to metabolic rate for various species during exercise. At a T_a of 20°C a 24 g mouse would be expected to dissipate 8.5 times its resting metabolic rate (*i.e.*, a total heat loss of ≈ 80 W/kg) passively through conduction, convection, and radiation without increasing evaporative heat loss. The 106 g hamster would be expected to dissipate 8.0 times its metabolic rate (*i.e.*, a total heat loss of ≈ 53 W/kg) with non-evaporative mechanisms before activating evaporative heat loss. Of course, it may not be possible to equate physically or physiologically the heat load from microwave exposure and exercise, however, the general relationship developed by Taylor does aid in explaining the large differences in threshold SAR between the mouse and hamster.

Acknowledgements

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References

- Aschoff, J. (1981). Thermal conductance in mammals and birds: its dependence on body size and circadian phase. *Comp. Biochem. Physiol.* 69A: 611-619.
- Cabanac, M. (1975). Temperature regulation. *Annu. Rev. Physiol.* 38: 415-439.
- Gordon, C. J. (1982). Effect of heating rate on evaporative heat loss in the microwave-exposed mouse. *J. Appl. Physiol.* 53: 316-323.
- Gordon, C. J. (1983a). Influence of heating rate on control of heat loss from the tail in mice. *Am. J. Physiol.* 244: R778-R784.
- Gordon, C. J. (1983b). Behavioral and autonomic thermoregulation in mice exposed to microwave radiation. *J. Appl. Physiol.* 55: 1242-1248.
- Gordon, C. J., M. D. Long and K. S. Fehlner (1983). Behavioral and autonomic thermoregulation in hamsters during microwave-induced heat exposure. *J. Thermal Biol.* (in press).
- Gordon, C. J. and J. S. Ali (1984). Measurement of breathing rate in unrestrained mice using microwave radiation. *Respir. Physiol.* 56: 73-79.
- Guieu, J. D. and J. D. Hardy (1970). Effects of heating and cooling of the spinal cord on preoptic unit activity. *J. Appl. Physiol.* 29: 675-683.
- Hammel, H. T. (1968). Regulation of internal body temperature. *Annu. Rev. Physiol.* 30: 641-710.
- Hart, J. S. (1971). Rodents. In: *Comparative Physiology of Thermoregulation*, edited by G. C. Whitrow. Academic Press, New York, pp. 1-149.
- Heath, J. E., B. A. Williams, S. H. Mills and M. J. Kluger (1972). The responsiveness of the preoptic-anterior hypothalamus to temperature in vertebrates. In: *Hibernation and Hypothermia, Perspectives and Challenges*, edited by F. E. South, J. P. Hannon, J. R. Willis, E. T. Peggelley and N. R. Alpert. Amsterdam, Elsevier, pp. 605-627.

- Hellstrom, B. and H. T. Hammel (1967). Some characteristics of temperature regulation in the unanesthetized dog. *Am. J. Physiol.* 213: 547-556.
- McEwen, G. N., Jr. (1973). Thermoregulation in the Unrestrained New Zealand White Rabbit, *Oryctolagus Cuniculus*. Ph.D. Thesis, University of Illinois, Urbana.
- McEwen, G. N., Jr., and J. E. Heath (1973). Resting metabolism and thermoregulation in the unrestrained rabbit. *J. Appl. Physiol.* 35: 884-886.
- McEwen, G. N., Jr., and J. E. Heath (1975). Thermal responses to preoptic heating and ambient temperature in unrestrained rabbits. *J. Thermal Biol.* 1: 23-27.
- Taylor, C. R. (1977). Exercise and environmental heat loads: different mechanisms for solving different problems? In: *International Review of Physiology*, V. 15, *Environmental Physiology II*, edited by D. Robertshaw. Baltimore, University Park Press, pp. 119-146.